AMENDMENTS TO THE SPECIFICATION

ON PAGE 1

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 21 AND CONTINUING TO LINE 27, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

Devices for manipulating fluids on the microscale have been developed to store, hold, and manipulate small amounts of fluids and have been applied to the detection of analytes in sample fluids. For example, capillary electrophoresis, generally involving the separation of charged species in solution, can be advantageously performed in a microchannel – see for example WO 96/04547, incorporated herein by reference. Electrokinetic and electroosmotic forces have been used to manipulate fluids in microfluidic devices, see WO 96/04547 for example. Manipulating fluids and performing capillary electrophoresis in [[an]]microfluidic devices promises advantages of small size, high throughput, low sample volumes, and cost.

ON PAGE 2

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 17 AND CONTINUING TO LINE 22, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

According to another aspect of the present invention, a method for ~~reseting~~ resetting a reservoir in a portable device for target analyte detection is provided. A first reservoir is placed in fluid communication with an inlet of a microfluidic chip. The first reservoir is removed from fluid communication with the microfluidic chip. A second reservoir is placed in fluid communication with the inlet of the microfluidic chip. Removing the first reservoir and placing the second reservoir maintains a contiguous fluid stream between an inlet of the microfluidic chip and a separation channel within the chip.

ON PAGE 2

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 23 AND CONTINUING TO LINE 30, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

According to another aspect of the present invention, a method for determining the presence of a target analyte in a sample using a portable device comprising a sample introduction port and an output interface is provided. A plurality of reservoirs [[are]]is coupled to a microfluidic chip within the portable device through a fluid manifold base. The input port is contacted with the sample. A microfluidic separation is performed according to a first separation characteristic within said portable device using at least a portion of said sample. A first separated component of the sample is detected, based on the microfluidic separation. The target analyte is identified, based on the detected component, and detection is indicated on the output interface.

ON PAGE 3 AND CONTINUING TO PAGE 4

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 34 AND CONTINUING TO LINE 13 ON PAGE 4, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

In some embodiments, a plurality of separations [[are]]is performed on the sample to enhance or verify the identification of the target analyte. In some embodiments, the portable device is modular and various components – including, for example, the detection module – can be removed and replaced between separations. Further, in some embodiments a plurality of samples can be analyzed sequentially, and/or simultaneously with the previous samples being stored in a waste reservoir, as is described further below. In some embodiments, the microfluidic separation is performed in a separation channel having a low-dispersion curve. Generally, as described further below, a low-dispersion curve limits the dispersion of a separated component as it traverses the separation channel, thereby enhancing the ability to accurately detect the component. In some embodiments, one or more reservoirs are in fluid communication with a microfluidic chip within the device through a fluid manifold base. This allows one or more reservoirs to be

removed and replaced without introducing gas to the microfluidic chip. A general description of a device having subsystems useful with embodiments of the present invention is also found in G.A. Thomas, et. al. "μChemLab™ - an integrated microanalytical system for chemical analysis using parallel gas and liquid phase microseparations" Proc. SPIE Vol. 3713, p. 66-76, Unattended Ground Sensor Technologies and Applications, Edward M. Carapezza; David B. Law; K. Terry Stalker; Eds., July 1999, hereby incorporated by reference in its entirety.

ON PAGE 10 AND CONTINUING TO PAGE 11

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 26 AND CONTINUING TO LINE 2 ON PAGE 11, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

In some embodiments, a reservoir comprises one or more chambers. In some embodiments, the chambers are in fluidic communication, however, in some embodiments fluids are confined to the individual chambers. In some embodiments, the chambers are in electronic communication[[,]]; however, in some embodiments the individual chambers are electronically isolated. In a preferred embodiment, a reservoir comprises two chambers separated by a barrier, such as, for example, an ion permeable membrane, salt bridge, dialysis membrane, polymer film, diffusion membrane, ionomer, e.g. ~~Nafion~~ NAFION® from Dupont, nanoporous glass, e.g. ~~Vycor~~ VYCOR® from Corning, and/or the like. In some embodiments, one chamber contains a fluid to be contacted with the microfluidic chip. A second chamber contains a fluid in contact with an electrode and is not in fluid communication with the microfluidic chip. The barrier permits electrical communication between the two chambers, in this embodiment, and prevents fluidic communication between the chambers. In this manner, fluid entering the microfluidic chip is not altered by any effects of applying a voltage across the fluid, such as pH change.

ON PAGE 15 AND CONTINUING TO PAGE 16

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 17 AND CONTINUING TO LINE 2 ON PAGE 16, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

In some embodiments, as described further below, the reservoir module further comprises an introduction port. In some embodiments, the introduction port is the sample introduction port for the portable device. In other embodiments, the fluid manifold introduction port is in fluid communication with a sample introduction port in an external housing, through tubing, channels, or other means known in the art, for example when a gaseous or aerosol sample is being collected. In some embodiments, a plurality of introduction ports [[are]]is provided. The introduction port, in some embodiments, is placed on one side of the fluid manifold base. One or more channels are provided in the fluid manifold base coupling the introduction port to one or more input ports on the microfluidic device. In preferred embodiments, the channel is as short as can be formed in the fluid manifold base to minimize the amount of fluid necessary to fill the channel. In a preferred embodiment, an injector port has a sample injector volume between the port and the microfluidic chip of less than 1500 nanoliters, more preferably less than 1000 nanoliters, still more preferably less than 500 nanoliters, and most preferably about 50-100 nanoliters. In a preferred embodiment, for example, the sample fluid containing a target analyte of interest is injected into the microfluidic device through the introduction port in the fluid manifold base. Embodiments of the introduction port can accommodate, for example, a standard syringe, tubing, pumps such as electrokinetic pumps, peristaltic pumps, hydrostatic pumps, displacement pumps, balloon, bladder, or any other injection mechanism as known in the art – including simply contacting the introduction port with a sample, such as by spitting. In some embodiments, an introduction port is provided in fluidic communication with a channel in the fluid manifold base that connects to one or more reservoirs in the reservoir module. Accordingly, in preferred embodiments, one or more reservoirs may be filled, refilled, or added to by injecting fluid into an introduction port.

ON PAGE 17

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 30 AND CONTINUING TO LINE 35, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

In one embodiment, the channel flush port is connected to a syringe and is in fluidic communication with a channel flush inlet and flush channel of the microfluidic chip. The channel flush port serves as [[a]]an access point for introducing the separation medium onto the microfluidic chip for the purpose of filling or exchanging the separation medium. In another embodiment, the channel flush port can serve as an access point for evacuating the separation medium from the microfluidic chip.

ON PAGE 20

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 28 AND CONTINUING TO LINE 32, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

Further electrophoretic media that may be used in conjunction with embodiments of the present invention may be found in U.S. Application Number 09/310,465, filed 12 May 1999 entitled "Castable 3-dimensional Stationary phase for ~~chromatorgraphy~~ chromatography" and U.S. Application Publication Number 2001/0008212 entitled "Castable Three-dimensional Stationary Phase for Electric Field-Driven Applications", filed 2/28/2001, both of which are hereby incorporated by reference.

ON PAGE 21

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 8 AND CONTINUING TO LINE 20, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

One embodiment of a microfluidic chip according to the present invention is shown in FIG. 2. Various channels are described and referenced, however it is to be understood that the channels are referred to according to their function, and, as is shown, several are contiguous. In general, microfluidic chip 200 contains inlets (or outlets) and channels, and/or chambers. Inlets/outlets allow access to the different reservoirs to which

they are connected for the purpose of introducing or removing fluids from the channels/chambers on the microfluidic chip 200. A contiguous fluid path through the inlet allows the passage of electrical current through conductive fluids. It will be understood that the number of inlets/outlets, channels/chambers, their size and configuration, placement, or other design or geometrical arrangement will vary according to the application contemplated on the microfluidic chip. In some embodiments, the configuration of the microfluidic chip will vary according to the physics and chemistry used to perform a microfluidic separation based on a particular analyte characteristic
– including, but not limited to, electrophoretic mobility, ~~molectular~~ molecular weight, hydrodynamic volume, isoelectric point, or partition coefficient.

ON PAGE 23 AND CONTINUING TO PAGE 24

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 31 AND CONTINUING TO LINE 2 ON PAGE 24, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

In some embodiments, a pressure stop is positioned between two or more channels in a microfluidic chip. For example, in some embodiments, a stop is positioned between the sample loop 212 and the sample channel 218, referring to FIG. 2. The sample loop is deeper than the sample channel. A first pressure is required to inject fluid into the sample loop. This pressure is not large enough, however, to force fluid into the sample channel. Accordingly, the sample channel (and other connected channels) are pressure-
– isolated [[form]]from the pressure injection.

ON PAGE 24 AND CONTINUING TO PAGE 25

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 30 AND CONTINUING TO LINE 7 ON PAGE 24, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

– Generally, optical detection of non-fluorescent target analytes ~~involve~~ involves providing a colored or luminescent dye as a 'label' on the target analyte. Fluorescent analytes may be directly detected by optical methods described below. Preferred labels include, but are not limited to, fluorescent lanthanide complexes, including those of

Europium and Terbium, fluorescein, fluorescamine, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue™, Texas Red, 1,1'-[1,3-propanediylbis[(dimethylimino-3,1-propanediyl]]bis[4-[(3-methyl-2(3H)-benzoxazolylidene)methyl]]-,tetraioide, which is sold under the name YOYO-1, Cy and Alexa dyes, and others described in the 9th Edition of the Molecular Probes Handbook by Richard P. Haugland, hereby expressly incorporated by reference. Labels may be added to 'label' the target analyte prior to introduction into the microfluidic chip, in some embodiments, and in some embodiments the label is added to the target analyte in the microfluidic chip. In general the labels are attached covalently as is known in the art, although non-covalent attachments may also be used.

ON PAGE 25 AND CONTINUING TO PAGE 26

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 10 AND CONTINUING TO LINE 4 ON PAGE 26, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

In a preferred embodiment, detection occurs using laser-induced fluorescence, as known in the art. Accordingly, in some embodiments, the detector module includes a light source, detector, and other optical components to direct light onto the microfluidic chip and collect fluorescent radiation from the target analyte. The light source preferably includes a laser light source, more preferably a laser diode, and still more preferably a violet or a red laser diode. A violet, or blue, laser diode is preferred in embodiments of the present invention to detect a fluorescamine label on one or more components of the sample. A fluorescamine label is preferred, in embodiments of the present invention, because the fluorescamine label attaches quickly (in milliseconds, in some embodiments) to the components of interest. Accordingly, fluorescamine is preferred in some embodiments to facilitate faster detection of one or more sample components. Violet, or blue, optical sources are accordingly preferred to excite the fluorescamine label. Other color laser diodes may be used, including red laser diodes, as well as other light sources including, but not limited to, laser diodes, light-emitting diodes, VCSELs, VECSELs, and

diode-pumped solid state lasers. In some embodiments, a Brewster's angle laser induced fluorescence detector is used. One or more beam steering mirrors are used, in one embodiment, to direct the beam to a detection area on the microfluidic chip. In preferred embodiments, the beam is directed onto the ~~micofluidic~~ microfluidic chip at Brewster's angle for the material of the chip. For example, in preferred embodiments the microfluidic chip comprises fused silica and the laser diode is directed onto the microfluidic chip at Brewster's angle for fused silica. Beam conditioning optics – including any of, but not limited to lenses, filters, and/or pinholes – may be used to focus the beam onto the microfluidic device. Dye may be injected into the microfluidic chip, in one embodiment, to visualize the location of the beam. A lens is used to collect and collimate the fluorescence and scattered light from the fluidic device. In embodiments where the microfluidic chip comprises a plurality of microchannels, each having a detection area, the detector module comprises a plurality of laser diodes (or other light sources), a plurality of beam steering mirrors to direct light from each diode to a microchannel. The collected light passes through a filter to remove the scattered laser light and the balance of the emissions are detected with a single photomultiplier tube for all channels. To eliminate cross-talk between the detection of each channel, electronics are used to alternately pulse each of the diode lasers so that fluorescence is only generated on one of the fluidic channels at any one time. In a preferred embodiment, the microfluidic chip includes 2 microchannels, and the detector comprises 2 laser diodes. However, any number of microchannels and laser diodes may be used, including, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 microchannels and a corresponding number of laser diodes.

ON PAGE 26

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 5 AND CONTINUING TO LINE 20, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

In a preferred embodiment, the detector module comprises a laser-induced fluorescence photometer, an example of which is shown in FIG. 3. In these embodiments,

a ~~colinear~~ collinear, or epifluoresence, optical configuration is used to deliver a focused beam of light to a microfluidic chip and collect and detect the fluorescence generated. In these embodiments, the detection module 300 includes a light source 302, as described above. The light source is placed into a variable x-y mount, in some embodiments, to facilitate alignment of the illumination with the separation channel. The laser light source may be collimated using collimating objective 304, most preferably an aspherical lens, and passed through an excitation filter 305 to reject laser diode emissions ~~oursside~~ outside the desired wavelength range. The collimated beam is reflected off a plurality of reflectors 306, with 4 dichroic reflectors being particularly preferred, to further condition the laser beam before passing into a beam steering block 309. The collimated beam is reflected off a dichroic reflector 308 and focused on the microfluidic chip with an objective 310. The same objective 310 collects the emissions from the microfluidic chip and the collimated emissions are passed through the dichroic folding mirror 308. Emissions are reflected off mirror 312, pass through emission filter 313, which rejects light that is not from fluorescence, in some embodiments. Fluorescence emissions impinge on a photodetector 314 and result in an electrical response, as known in the art.

ON PAGE 29

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 1 AND CONTINUING TO LINE 24, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

A microfluidic separation is performed, as known in the art. The particular procedure for performing a microfluidic separation will vary according to the type of separation performed and the microfluidic chip configuration. In one embodiment, the separation proceeds as follows, with reference to FIG. 2, a voltage and current are applied to the sample electrode and sample waste electrode positioned in the sample reservoir and sample waste reservoir, respectively. Referring back to FIG. 2, sample in the sample loop channel moves under the influence of the electric field and fills the sample channel, injection cross and begins to fill the sample waste channel. In one embodiment, a smaller voltage and current is applied to the buffer electrode and waste electrode, positioned in

the buffer reservoir and waste reservoir, respectively, to help confine sample in the injection cross; a pinched injection as is known by those familiar with the art. The voltages and currents causing the electrokinetic injection are turned off. A voltage and current are applied to the buffer electrode and waste electrode, positioned in the buffer reservoir and waste reservoir, respectively, in one embodiment. The sample contained in the injection cross moves into the separation channel and begins to divide into individual analyte zones. In one embodiment, a smaller voltage and current are applied to the sample electrode and sample waste electrode to prevent sample from spilling from the sample channel and sample waste channel into the injection cross and the separation channel; ~~anti-siphioning~~ <u>also known as an anti-siphoning</u> voltage ~~as is known~~ by those familiar with the art. The separation voltage is applied until the individual analyte zones pass through the separation channel, past the detection area and into the waste channel. The time between the application of the separation voltage and the appearance of the center of the analyte zone in the detector signal defines the time for the analyte in the sample, in one embodiment, and is indicative of the presence of the analyte in the sample. Time may be converted into a characteristic for the component, such as electrophoretic mobility, molecular weight, hydrodynamic volume, isoelectric point, or partition coefficient, in some embodiments to facilitate determination of the component and/or analyte. The analysis process may be repeated by injection of a second sample into the sample loop channel, in some embodiments.

ON PAGE 30

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 13 AND CONTINUING TO LINE 22, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

In embodiments where a plurality of microchannels are provided on a microfluidic chip, a second portion of the sample fluid may be transported to a second separation channel, and a detection area on the second separation channel is interrogated with the detection module. In some embodiments, a plurality of microfluidic chips [[are]]<u>is</u> provided, each with one or more separation channels. A single sample may be injected

and multiplexed onto each chip, in one embodiment. In another embodiment, separate samples, or portions of a single sample, are injected, one into each microfluidic chip. In some embodiments where a plurality of microfluidic chips are provided, one or more microfluidic chips are configured to perform the same or different microfluidic separation method and, where one or more samples are introduced into the device, the distribution of samples among microfluidic separation methods can be in any association.

ON PAGE 30 AND CONTINUING TO PAGE 31

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 30 AND CONTINUING TO LINE 10 ON PAGE 31, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

The microchip was fabricated from Corning 7980 fused silica wafers (100mm diameter, 0.75 mm thickness using standard photolithography, wet etch, and bonding techniques. Fused Silica wafers were PECVD deposited with amorphous silicon (150 nm), which served as the hard mask. A 7.5-micron thick layer of positive photoresist was spin-coated and soft-baked (90C, 5 minutes). The mask pattern was transferred to the photoresist by exposing it to UV light in a contact mask aligner. After exposure, the photoresist was developed and hard-baked (125C, 30 minutes). Exposed silicon was etched in a plasma etch tool.**[[.]]** Silicon etch process consisted of a 30 second oxygen ash @ 200W DC & 25 mTorr, followed by 150 second SF6 @ 200W DC & 50 mTorr. The subsequently exposed glass was etched with a 49% HF solution. Via access holes were drilled in the cover plate (Corning 7980) with diamond-tipped drill bits. The etched wafers and drilled cover plates were cleaned with 4:1 H2SO4:H2O2 (100C), de-stressed with 1% HF solution, then the surfaces were treated in 80C 40% NaOH, rinsed in a cascade bath, followed by a spin rinse dry, aligned for contacting, and thermally bonded at 1150C for 5 hours in an N2-purged programmable muffle furnace. The standard chips were cut with a programmable dicing saw containing a diamond composite blade into 25.4 x 25.4mm or 20x 20mm devices depending upon design.

ON PAGE 33

IN THE ORIGINAL PARAGRAPH BEGINNING AT LINE 23 AND CONTINUING TO LINE 32, PLEASE AMEND THE SPECIFICATION AS FOLLOWS:

– Referring now to FIG 9, ~~illustrating~~ which illustrates the analysis scheme used by the device, generally, [[. A]]a sample to be analyzed is injected into a receiving means that can include introduction port 32 and at least one of reservoirs 65, here sample reservoir 65a. A portion of the injected sample is drawn into the flow channel 74 leading from the sample reservoir 65a to waste reservoir 65b by application of a voltage between electrode 72a, contained within sample reservoir 65a and electrode 72b contained within sample waste reservoir 65b. As a result of this operation, a portion of the sample flowing in channel 74 between the sample and sample waste reservoirs resides in the Z-bend in channel 74 (FIG 12 inset). This portion forms an injection plug which is subsequently injected into separation channel 75 by application of a voltage between electrode 72c contained within buffer reservoir 65c and electrode 72d contained within waste reservoir 65d. The volume of the injected is determined by the dimensions of the Z-bend.